

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

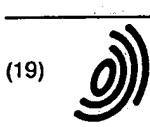
Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.



(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 759 775 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
26.07.2000 Bulletin 2000/30

(51) Int. Cl. 7: **A61K 38/21**

(21) Application number: **95921749.8**

(86) International application number:
PCT/EP95/01825

(22) Date of filing: **15.05.1995**

(87) International publication number:
WO 95/31213 (23.11.1995 Gazette 1995/50)

(54) IFN-BETA LIQUID FORMULATIONS

FLÜSSIGE BETA-INTERFERON FORMULIERUNGEN
FORMULATIONS LIQUIDES D'INTERFERON BETA

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE**

(30) Priority: **16.05.1994 IT RM940300**

(43) Date of publication of application:
05.03.1997 Bulletin 1997/10

(73) Proprietor:
**Applied Research Systems ARS Holding N.V.
Curacao (AN)**

(72) Inventors:
• **SAMARITANI, Fabrizio
I-00139 Rome (IT)**
• **NATALE, Patrizia
I-00199 Rome (IT)**

(74) Representative:
**Welsert, Annekäte, Dipl.-Ing. Dr.-Ing.
Patentanwälte
Kraus & Welsert
Thomas-Wimmer-Ring 15
80539 München (DE)**

(56) References cited:
**WO-A-89/02750 US-A- 4 455 622
US-A- 5 183 746**

• Dialog Information Services, file 351, WPIL,
Dialog accession no. 003925794, WPI accession
no. 84-071338/12, TORAY IND INC: "Stabilising
beta-Interferon having no sugar chain by adding
polyol, e.g. ethylene glycol, glycerine or sugar,
esp. oligo saccharide"; & JP,A,59025333,
840209, 8412 (Basic)

EP 0 759 775 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description

[0001] The present invention relates to liquid formulations of interferon-beta (IFN- β) stabilised with a polyol selected from mannitol, saccharose and glycine, a non-reducing sugar or an amino acid. In particular, it relates to liquid formulations containing mannitol human albumin and acetate buffer.

[0002] Interferons (alpha, beta, gamma) are glycoproteins produced in the cells of vertebrates following induction. The most traditional inducers are virus, but also other microbial agents, other natural substances and synthetic compounds have the same behaviour.

[0003] Interferon- β is induced in human fibroblasts, has anti-viral activity, but in the therapy of some tumoral forms other activities can be exploited together with the anti-viral, such as the anti-proliferative cellular activity and immunoregulatory activity.

[0004] Production from culture of human fibroblasts, and specifically from recombinant DNA techniques, now allows to obtain industrial quantities of interferon-beta.

[0005] It is known that proteins in the purified form are especially susceptible to degradation, even due to the normal activity of atmospheric agents. This peculiarity becomes even more evident for proteins produced according to recombinant DNA techniques.

[0006] As a direct consequence of the fact that highly purified proteins are easily subject to denaturation, it becomes desirable to obtain stable formulations which ensure the longest possible life-cycle to the product.

[0007] Stabilisation of formulations containing highly purified proteins may be carried out by the addition of one or more excipients which inhibit or delay degradation of the active principle.

[0008] Pharmaceutical compositions containing interferon-beta are well known. EP Patent application 89 245 (INTER-YEDA Ltd) describes a lyophilised composition of interferon-beta containing mannitol, human albumin and polyvinylpyrrolidone, the latter as stabilising agent. Also known are pharmaceutical liquid compositions containing other interferons.

[0009] International Patent Application WO 89/04177 (GENENTECH - Priority 03/11/87) describes liquid pharmaceutical formulations of gamma-interferon comprising a buffer which maintains the pH within the range of 4.0-6.0, a polyhydroxylate sugar as stabiliser and a non-ionic detergent.

[0010] EP Patent Application 270 799 describes IFN- β pharmaceutical compositions in liquid form or lyophilized, which comprise, as solubilizer/stabilizer, one or more non-ionic polymeric detergents.

[0011] It is highly desirable to obtain such liquid formulations in order to avoid the reconstitution of lyophilised preparations and thus to permit ease of use.

[0012] It has now surprisingly been found that liquid pharmaceutical formulations comprising interferon-beta stabilised with a polyol selected from mannitol, saccharose and glycine, a non-reducing sugar or an amino acid in an appropriate buffer are particularly stable and maintain biological activity for a long period of time.

[0013] The main object of the present invention is to provide a liquid pharmaceutical formulation comprising interferon-beta and a polyol selected from mannitol, saccharose and glycine, a non-reducing sugar or an amino acid, as stabiliser.

[0014] Preferably the stabiliser is mannitol.

[0015] Preferably the liquid pharmaceutical formulation comprises a buffer with a pH between 3 and 4; more preferably, acetate buffer.

[0016] Another object of this invention is to provide a process for the preparation of such liquid pharmaceutical formulation comprising the stage of dilution of IFN- β with a solution of the excipients.

[0017] Yet another object of the present invention is to provide a presentation form of the liquid pharmaceutical formulation comprising the previously mentioned formulation, hermetically sealed under sterile conditions in a container suitable for storage prior to use.

[0018] To study the stability of liquid formulations of IFN- β , various formulations were prepared diluting bulk IFN- β in different buffers at varying pH, then storing the samples at different temperatures and carrying out assays with the immunological test at set intervals of time. Once the buffer solution and the preferred pH, with which the greater stability is obtained, have been selected, then the stabilised formulations of the invention are prepared by diluting the interferon bulk solution with the buffer solution containing also the excipients. Stability of the various formulations was determined by measuring the residual activity of IFN- β at fixed intervals of time, after storage of the solution at the temperatures of 50°C, 37°C, and 25°C.

[0019] To determine such activity, samples were assayed under immunological and biological tests.

[0020] The immunological test was carried out by using the TORAY kit (Human IFN-Beta ELISA Kit, TORAY INDUSTRIES, Inc.), following the methodology reported in the enclosed instructions.

[0021] The biological dosage was performed as described by Armstrong J.A. (1981), Cytopathic effect inhibition assay for Interferon, in Methods in Enzymology 73 381-387. This test permits the measuring of IFN- β activity by exploiting its anti-viral capacity.

[0022] Measure of activity is expressed in International Units per millilitre of solution (IU/ml) or in Mega International Units per millilitre of solution (MIU/ml). (1 MIU/ml = 1,000,000 IU/ml).

[0023] An International Unit is calculated as described in the Research Reference Reagent Note No. 35, published by the National Institute of Health, Bethesda, Maryland, in relation to the HuIFN-beta NIH Reference Reagent Gb 23-902-531 used as standard.

5 [0024] The measurement is reported here as percentage of residual activity of the sample of Interferon-beta in the various formulations, taking activity of the sample at time zero as equal to 100%.

[0025] Dosages were carried out in duplicate.

10 [0026] To assess the effect of the pH on stability of the active ingredient, different formulations of recombinant IFN- β were prepared containing 0.6 and 1 MIU/ml with various buffer solutions, i.e. acetate buffer, citrate buffer, ascorbate buffer, succinate buffer.

[0027] The formulations containing recombinant IFN- β with the buffer solutions were prepared and stored at temperatures of 50°C, 37°C and 25°C, then assayed under the immunological test at set time intervals. The formulations were prepared in such a way as to have a pH between 3.0 and 4.0 and between 5.0 and 6.0, all with buffer at a concentration of 0.01 M.

15 [0028] Tables 1, 2 and 3 report results of tests carried out at set intervals of time, from 1 to 42 days, at the various temperatures.

[0029] Data contained in the above-mentioned tables indicate that the formulations with a pH between 5.0 and 6.0 show an immediate loss of titre. Formulations with pH between 3.0 and 4.0 show, however, a high stability, especially in 20 the presence of acetate buffer.

[0030] To assess the effect of excipients on the stability of the active principle, different formulations were prepared containing 1 MIU/ml of recombinant IFN- β , using various excipients such as mannitol, saccharose or an amino acid such as glycine, and human albumin already partially contained in the interferon-beta bulk solution.

25 [0031] Quantities of mannitol, saccharose or glycine used were such as to obtain isotonic solutions of IFN- β .

[0032] Stability studies on these formulations were carried out by maintaining samples at 50°C, 37°C, 25°C and 4°C, and measuring residual activity at the times reported in tables 4 and 5.

[0033] Data reported in tables 4 and 5 show that degradation in the formulations containing a polyol like mannitol is much lower in respect to those formulations containing saccharose or glycine.

[0034] The formulation selected for a deeper study was the one containing mannitol in 0.01 M acetate buffer at pH 30 3.5, which was subjected to further tests for evaluation of the effect on stability of the ionic force and the albumin.

[0035] Solutions of IFN- β in 0.01 M acetate buffer at pH 3.5, were prepared at different values of osmolality: 150, 300 and 400, and with different dielectric constants, with 5, 10, and 20 % propylene glycol, and samples were then stored and assayed at 50°C, 37°C and 25°C. The study shows that increase of osmolality and the propylene glycol content decreased stability of the liquid formulations of IFN- β .

35 [0036] Since bulk IFN- β contains albumin, it was decided to proceed to a study for evaluation of the effect of albumin on the stability of interferon- β liquid formulations. Samples containing IFN- β (1 MIU/ml) and the acetate buffer solution at pH 3.5 were added to 1, 3, 6, and 9 mg/ml of human albumin and tested at temperatures of 50°C, 37°C and 25°C.

[0037] Results show that with the increase of albumin the stability of the samples decreased. The albumin content per sample was fixed in such a way as to have the minimum quantity compatible with that contained in the various bulks: 40 in a formulation containing 1 MIU/ml of IFN- β , a uniform content of 0.5 mg/ml albumin is maintained.

EXAMPLES of PHARMACEUTICAL PRODUCTION

[0038] Materials: mannitol (Merck); human albumin (Boehringer); 0.01 M acetate buffer (Merck); NaOH 1M (Merck).
45 DIN 2R glass bottles (glass type I borosilicate glass) with stoppers of Pharmagummi rubber, butylc mixture, and aluminium ring, were used as containers.

Example of preparation of r-IFN- β solution

50 A) SOLUTION AT 1 MIU/ML

[0039] For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

55

r-interferon-beta	1000 MIU
Mannitol	54.6 g

(continued)

5	Human albumin	0.5 g·P
	0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.
P = amount of human albumin present in bulk interferon.		

B) SOLUTION AT 12 MIU/ML

10 [0040] For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

15	r-interferon-beta	12000 MIU
	Mannitol	54.6 g
20	Human albumin	4.0 g·P
	0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.
P = amount of human albumin present in bulk interferon.		

C) SOLUTION AT 24 MIU/ML

25 [0041] For the preparation of a batch of 1 Lt. of finished product, the following quantities are used:

30	r-interferon-beta	24000 MIU
	Mannitol	54.6 g
35	Human albumin	8.0 g·P
	0.01 M pH 3.5 acetate buffer	q.s. to 1 Lt.
P = amount of human albumin present in bulk interferon.		

Method of Preparation

40 [0042] The required quantity of mannitol and human albumin (taking into account the quantity of albumin present in the bulk) is dissolved in approximately 500 g of 0.01 M pH 3.5 acetate buffer. The pH is checked and, if necessary, 45 adjusted to the value of 3.5 ± 0.2 with diluted (1:2) acetic acid or with 1 M NaOH.

[0043] The solution is brought to the final weight of 1 Kg with 0.01 M pH 3.5 acetate buffer.

[0044] The required quantity of r-interferon beta is weighed in a beaker and brought to the final weight of 500 g with the solution of excipients.

50 [0045] In another beaker 500 g of solution of excipients is weighed. The 500 g of solution containing interferon-beta is filtered on a sterile membrane of 0.22 μm (DURAPORE) at a pressure not exceeding 1.5 atm. The sterile solution is collected in a glass erlenmeyer flask. Immediately afterwards the 500 g of excipient solution is filtered on the same membrane at a pressure of 1.5 atm and collected in the same erlenmeyer flask. The solution obtained is slowly mixed.

5

TABLE 1
r-INTERFERON-β

10 **LIQUID FORMULATION : 1 MIU/BOTTLE**
 15 **RESULTS IMMUNOLOGICAL DOSAGE :**
CONCENTRATION (%)
STABILITY IN 0.01 M CITRATE BUFFER AT
DIFFERENT pH VALUES

20

	50°C		37°C		25°C		
	1 → 0	1.D	30.D	19.D	39.D	12.D	19.D
IFN/3	100 (890600 IU/ML)	73.0	8.6	88.5	69.9	100	97.5
IFN/4	100 (820900 IU/ML)	20.1	1.0	68.7	36.6	100	62.8
IFN/5	100 (532100 IU/ML)	ND					43.6
IFN/6	100 (179500 IU/ML)	ND					

35 D = day(s)

ND = non-determinable

40 IFN/3 = formulation in citrate buffer pH 3.0

IFN/4 = formulation in citrate buffer pH 4.0

IFN/5 = formulation in citrate buffer pH 5.0

45 IFN/6 = formulation in citrate buffer pH 6.0

5

TABLE 2
r-INTERFERON-8

10 LIQUID FORMULATION : 0.6 MIU/BOTTLE
RESULTS IMMUNOLOGICAL DOSAGE :
CONCENTRATION (%)
15 STABILITY IN 0.01 M ACETATE BUFFER AT DIFFERENT
pH VALUES

20

	100	40°C		57°C		25°C	
		1 D	10 D	19 D	39 D	7 D	19 D
IFN/3	100 (549425 IU/ML)	72.6	48.3	97.5	100	100	100
IFN/4	100 (459600 IU/ML)	77.6	30.3	91.9	92.2		100
IFN/5	100 (52275 IU/ML)	45.0					
IFN/6	100 (25425 IU/ML)	57.2					

40 D = day(s)
IFN/3 = formulation in acetate buffer pH 3.0
IFN/4 = formulation in acetate buffer pH 4.0
45 IFN/5 = formulation in acetate buffer pH 5.0
IFN/6 = formulation in acetate buffer pH 6.0

5

10

15

20

25

30

35

40

45

50

55

TABLE 3
r-INTERFERON- β

LIQUID FORMULATION : 1 MIU/BOTTLE
RESULTS IMMUNOLOGICAL DOSAGE : CONCENTRATION (%)
STABILITY IN 0.01 M ASCORBATE & SUCCINATE BUFFER AT pH 3.00 & 4.00

	1 = 0	50°C			57°C			25°C		
		14 D	21 D	28 D	14 D	21 D	28 D	14 D	21 D	28 D
IFN3/ASC	100 (1068400 IU/ML)	ND			32.4			76.5	10.5	
IFN4/ASC	100 (1025000 IU/ML)	ND			15.6			80.6		
IFN3/SUC	100 (980200 IU/ML)	62.9	54.8	22.1	92.7	87.8	96.0	62.5	97.2	
IFN4/SUC	100 (957600 IU/ML)	62.8	43.8	22.7	88.5	14.3		78.7	84.7	

D = days

ND = non-determinable

IFN3/ASC = formulation in ascorbic buffer pH 3.0;
IFN3/SUC = formulation in succinate buffer pH 3.0;IFN4/ASC = formulation in ascorbic buffer pH 4.0;
IFN4/SUC = formulation in succinate buffer pH 4.0

5
TABLE 4
 α -INTERFERON-8

10
LIQUID FORMULATION : 1 MIU/BOTTLE
RESULTS BIOLOGICAL DOSAGE : CONCENTRATION
(%)
15 STABILITY IN 0.01 M ACETATE BUFFER AT pH 3.5 with
DIFFERENT EXCIPIENTS

	40°C		49°C		55°C		
	1 - D	49 D	49 D	3 M	3 M	6 M	9 M
ACE/SAC/3.5	100 (970000 IU/ML)	25.8	100	85.6	100	100	
ACE/MAN/3.5	100 (1150000 IU/ML)	67.8	100	90.4	91.3	100	85.2
ACE/GLY/3.5	100 (1200000 IU/ML)	49.2	100	75.8	90.0	98.3	

40 D = days

M = months

45 ACE/ SAC /3.5 = formulation in acetate buffer pH 3.5 + saccharose

ACE/MAN/3.5 = formulation in acetate buffer pH 3.5 + mannitol

ACE/ GLY /3.5 = formulation in acetate buffer pH 3.5 + glycine

5
10
15
20
25
30
35
40
45
50
55

TABLE 5
r-INTERFERON- β

LIQUID FORMULATION : 1 MIU/BOTTLE
RESULTS IMMUNOLOGICAL DOSAGE : CONCENTRATION (%)
STABILITY USING DIFFERENT EXCIPIENTS IN 0.01 M ACETATE BUFFER AT pH 3.5

	100	50%			25%			10%		
		D	M	D	D	M	M	M	M	M
ACE/SAC /3.5	100 (1120000 IU/ML)	78.6	62.5	10.3	99.1	96.4	67.1	97.2	100	100
ACE/MAN/3.5	100 (1070000 IU/ML)	90.6	74.8	60.3	100	100	100	100	100	100
ACE/GLY /3.5	100 (1220000 IU/ML)	77.0	47.5	19.4	100	91.0	93.9	96.9	87.7	100

D = days
M = months

ACE/ SAC /3.5 = formulation in acetate buffer pH 3.5 + sucrose
ACE/MAN/3.5 = formulation in acetate buffer pH 3.5 + mannitol
ACE/ GLY /3.5 = formulation in acetate buffer pH 3.5 + glycine

Claims

1. A stable, liquid pharmaceutical formulation comprising interferon- β , a stabilising amount of a polyol selected

from mannitol, saccharose and glycine, and a buffer capable of maintaining the pH of the formulation at a value between 3.0 and 4.0.

2. A liquid pharmaceutical formulation according to claim 1, wherein the polyol is mannitol.
- 5 3. A liquid pharmaceutical formulation according to any of the claims 1 or 2, in which interferon-beta is recombinant.
4. A liquid pharmaceutical formulation according to any of the preceding claims, in which interferon-beta is in a quantity between 0.6 and 1 MIU/ml.
- 10 5. A liquid pharmaceutical formulation according to any of the preceding claims, in which the buffer solution is acetate buffer.
6. A liquid pharmaceutical formulation according to claim 4, in which the buffer solution has a concentration of 0.01 M.
- 15 7. A liquid pharmaceutical formulation according to any of the claims from 1 to 6, which also comprises human albumin.
8. A liquid pharmaceutical formulation according to any of the claims from 1 to 7, comprising 1 MIU/ml of interferon-beta, 54.6 mg/ml of mannitol, 0.5 mg/ml of albumin in a solution of 0.01 M acetate buffer at pH 3.5.
- 20 9. Process for the preparation of a liquid pharmaceutical formulation according to any of the claims from 1 to 8, comprising the dilution of interferon-beta with a solution of excipients.
- 25 10. A container hermetically sealed in sterile conditions comprising the liquid pharmaceutical formulation according to any of the claims from 1 to 8 and appropriate for storage prior to use.

Patentansprüche

- 30 1. Stabile, flüssige pharmazeutische Formulierung umfassend Interferon-beta, eine stabilisierende Menge eines Polyols, ausgewählt aus Manitol, Saccharose und Glycin und einen Puffer, der den pH-Wert der Formulierung auf einem Wert zwischen 3,0 und 4,0 halten kann.
2. Flüssige pharmazeutische Formulierung nach Anspruch 1, wobei das Polyol Manitol ist.
- 35 3. Flüssige pharmazeutische Formulierung nach einem der Ansprüche 1 oder 2, wobei das Interferon-beta rekombinant ist.
4. Flüssige pharmazeutische Formulierung nach einem der vorstehenden Ansprüche, bei der Interferon-beta in einer Menge zwischen 0,6 und 1 MIU/ml vorliegt.
- 40 5. Flüssige pharmazeutische Formulierung nach einem der vorstehenden Ansprüche, bei der die Pufferlösung ein Acetatpuffer ist.
6. Flüssige pharmazeutische Formulierung nach Anspruch 4, bei der die Pufferlösung eine Konzentration von 0,01 M aufweist.
- 45 7. Flüssige pharmazeutische Formulierung nach einem der Ansprüche 1 bis 6, welche außerdem humanes Albumin umfaßt.
8. Flüssige pharmazeutische Formulierung nach einem der Ansprüche 1 bis 7, umfassend 1 MIU/ml Interferon-beta, 54,6 mg/ml Manitol, 0,5 mg/ml Albumin in einer Lösung von 0,01 M Acetatpuffer, PH 3,5.
- 50 9. Verfahren zur Herstellung einer flüssigen pharmazeutischen Formulierung nach einem der Ansprüche 1 bis 8, umfassend die Verdünnung von Interferon-beta mit einer Trägermittellösung.
10. Unter sterilen Bedingungen hermetisch verschlossener Behälter, umfassend die flüssige pharmazeutische Formulierung nach einem der Ansprüche 1 bis 8, der für eine Lagerung vor Verwendung geeignet ist.

Revendications

1. Formulation pharmaceutique liquide stable comprenant de l'interféron bêta, un polyol en une quantité stabilisante, choisi parmi le mannitol, le saccharose et la glycine, et un tampon capable de maintenir le pH de la formulation à une valeur comprise entre 3,0 et 4,0.
5
2. Formulation pharmaceutique liquide selon la revendication 1, dans laquelle le polyol est le mannitol.
3. Formulation pharmaceutique liquide selon l'une quelconque des revendications 1 et 2, dans laquelle est l'interféron bêta est recombinant.
10
4. Formulation pharmaceutique liquide selon l'une quelconque des revendications précédentes, dans laquelle l'interféron bêta est présent en une quantité comprise entre 0,6 et 1 MUI/ml.
- 15 5. Formulation pharmaceutique liquide selon l'une quelconque des revendications précédentes, dans laquelle la solution tampon est un tampon d'acétate.
6. Formulation pharmaceutique liquide selon la revendication 4, dans laquelle la solution tampon a une concentration de 0,01 M.
20
7. Formulation pharmaceutique liquide selon l'une quelconque des revendications 1 à 6, comprenant aussi de l'albumine humaine.
8. Formulation pharmaceutique liquide selon l'une quelconque des revendications 1 à 7, comprenant 1 MUI/ml d'interféron bêta, 54,6 mg/ml de mannitol et 0,5 mg/ml d'albumine dans une solution d'un tampon d'acétate 0,01 M à pH 3,5.
25
9. Procédé de préparation d'une formulation pharmaceutique liquide selon l'une quelconque des revendications 1 à 8, comprenant la dilution de l'interféron bêta avec une solution d'excipients.
30
10. Conteneur scellé hermétiquement dans des conditions stériles, comprenant la formulation pharmaceutique liquide selon l'une quelconque des revendications 1 à 8 et qui est approprié à un stockage avant l'utilisation.

35

40

45

50

55